Supporting Information

for

Synthesis and Sensing Applications of A New Fluorescent Derivative of Cholesterol

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Experimental

Synthesis of I-1

To a solution of diethanolamine (5.00 g, 48 mmol) in $CHCl_3$ (50 mL), $SOCl_2$ (190 mmol, 14 mL) in $CHCl_3$ (40 mL) was added dropwise at 0 °C. Then, the mixture was stirred at room temperature for 3 h, and then further refluxed for 4 h. After evaporation of the excessive $SOCl_2$, the residue was purified by crystallization from ethanol to give 6.0 g (70%) of compound **1** as a white solid.

Synthesis of I-2

To a stirred solution of compound 1 (0.63 g, 3.5 mmol) and TEA (1500 μL, 10.5 mmol) in $CHCl_3$ (20 mL), cholesterol chloroformate (1.57 g, 3.5 mmol) in $CHCl_3$ (15 mL) was added dropwise at 0 °C for 3 h, and the mixture was stirred for 5 h at room temperature. Then, the reaction mixture was washed with 0.01 mol/L hydrochloric acid (50 mL × 3) and pure water (50 mL × 3), respectively. The organic layer was separated and filtered through a pad of anhydrous $MgSO_4$. After evaporating the excessive $CHCl_3$, the residue was purified by crystallization from ethanol to give 1.75 g (90%) of compound 2 as a white solid with m.p. 111.7–112.0 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 5.38 (s, 1H), 4.50-4.58 (m, 1H), 3.63-3.67 (m, 8H), 0.68-2.40 (m, 43H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ= 155.31, 155.29, 139.53, 122.74, 76.73, 75.53, 56.68, 56.15, 50.01, 42.31, 41.93, 39.73, 39.52, 38.52, 36.97, 36.56, 36.19, 35.79, 31.91, 31.87, 28.23, 28.18, 28.00, 24.29, 23.85, 22.82, 22.57, 21.06, 19.36, 19.25, 18.72, 11.86. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 554.3453, Found: 554.3450.

Synthesis of I-3

Compound 2 (1.00 g, 1.80 mmol) was dissolved in DMF (10 mL). The solution was stirred and NaN_3 (0.12 g, 1.80 mmol) was added. The mixture was stirred at 85 °C for 48 h, then the mixture was poured into water (20 mL), and then filtered. Finally, the filtrate was washed with $CHCl_3$ (20 mL × 3) and dried in vacuum to give compound 3 as canary powder, and then purified by silica gel chromatography (3:1 DCM/petroleum ether) to afford compound 3 (0.35g, 35%) as a white solid with m.p. 88.2-89.2 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 5.39 (s, 1H), 4.53-4.58 (m, 1H), 3.44-3.51 (m, 8H), 0.68-2.40 (m, 43H). IR (KBr, cm^{-1}): 2982, 2772, 2110, 1745. MS

(ESI, m/z): Calcd for [$(M+H)^+$]: 561.3857, Found: 561.3821.

Synthesis of I-4

$CuSO_4$ (0.19 g, 1.18 mmol) and sodium ascorbate (0.29 g, 1.48 mmol) were added to a solution of compound 3 (0.83 g, 1.48 mmol) and compound 7 (0.69 g, 1.48 mmol) in THF/H_2O (1:1) 32 mL. The mixture was stirred at room temperature for 18 h, and then filtered. The THF was removed under vacuum. The organic phase was washed with $CHCl_3$ and dried (Na_2SO_4). The residue was purified by silica gel chromatography (2:1 EtOAc /methanol) to afford compound 5 (1.11g, 73%) as a white solid with m.p. 165.3-166.6 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 7.54 (s, 1H), 5.38-5.39 (t, 2H), 5.24 (s, 1H), 4.42-4.56 (m, 6H), 3.25-3.80 (m, 6H), 0.68-2.36 (m, 86H). IR (KBr, cm^{-1}): 2982, 2772, 1745. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 1028.7620, Found: 1028.7651.

Synthesis of I-5

The synthetic procedures for compound 5 are similar to those for compound 3. The crude product was dried in vacuum to give a canary powder, and then purified by silica gel chromatography (2:1 EtOAc/petroleum ether) to afford compound 5 (0.38 g, 90%) as a white solid with m.p. 134.9-135.1 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 7.53 (s, 1H), 5.37-5.40 (t, 2H), 5.22 (s, 1H), 4.42-4.56 (m, 6H), 3.11-3.77 (m, 6H), 0.67-2.41 (m, 86H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ= 139.81, 139.50, 122.97, 122.66, 76.80, 75.87, 74.86, 56.81, 56.78, 56.27, 50.14, 50.10, 42.41, 39.84, 39.61, 38.63, 38.56, 37.10, 37.04, 36.64, 36.29, 35.89, 32.00, 31.96, 28.32, 28.25, 28.09, 24.38, 23.94, 22.90, 22.65, 21.17, 21.14, 19.46, 19.40, 18.82, 18.81, 11.96, 11.95. IR (KBr, cm^{-1}): 2982, 2772, 2110, 1745. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 1034.8024, Found: 1034.7998.

Synthesis of I-6

To a stirred solution 4-Chloro-7-nitro-1,2,3-benzoxadiazole of (1.42 g, 7.14 mmol) in EtOAc (50 mL), propargylamine (500 μL, 7.14 mmol) was added dropwise, and the mixture was stirred for 5 h at room temperature, and then filtered. The crude product was dried in vacuum to give a crimson powder, and then purified by silica gel chromatography (DCM) to afford orange powder (1.38 g, 89%) with m.p. 84.9-85.8

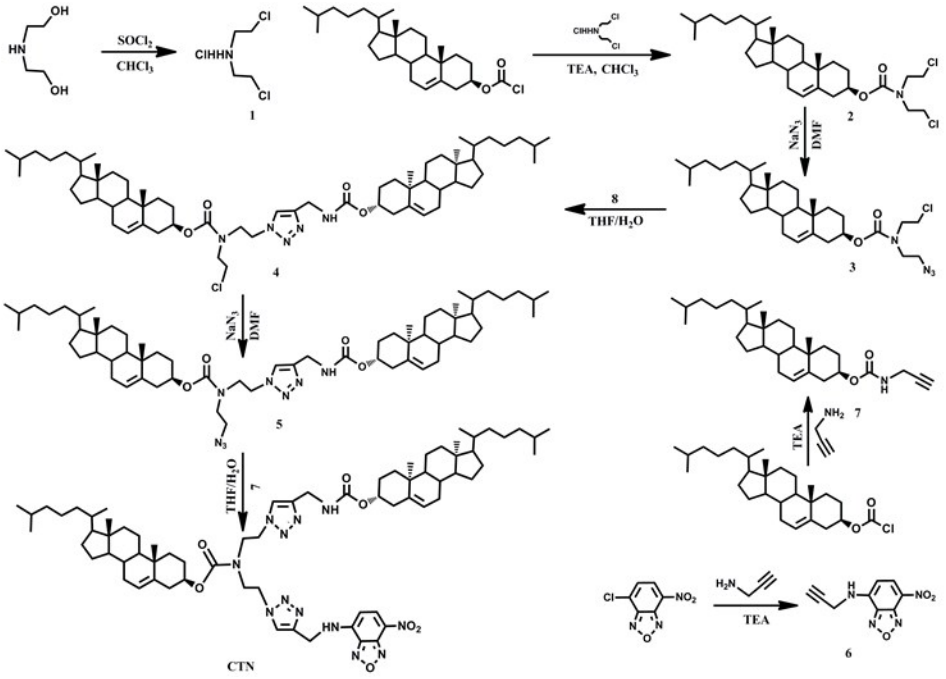
°C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 8.51 (d, 1H), 6.35-6.37 (d, 2H), 4.31 (d, 2H), 2.48 (d, 1H), IR (KBr, cm^{-1}): 3474, 3210, 2110, 1672. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 218.0440, Found: 218.0401

Synthesis of I-7

To a stirred solution of propargylamine (500 μL, 7.14 mmol) and TEA (2.00 mL, 14.29 mmol) in DCM (40 mL), cholesterol chloroformate (3.21 g, 7.14 mmol) in DCM (20 mL) was added dropwise at 0 °C for 3 h, and the mixture was stirred for 5 h at room temperature, and then filtered. Finally, the filtrate as washed with 0.01 mol/L hydrochloric acid (50 mL × 3) and pure water (50 mL × 3), respectively. The organic phase was dried with Na_2SO_4. The crude product was purified by silica gel chromatography (DCM) to afford compound 7 (2.67 g, 80%) as a white solid with m.p. 108.1-108.5 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 5.38 (s, 1H), 4.93 (s, 1H, -NH), 4.45-4.60 (m, 1H), 3.97 (s, 2H), 0.68-2.36 (m, 43H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ= 155.57, 139.70, 122.62, 79.86, 74.95, 71.46, 56.70, 56.16, 50.02, 42.33, 39.75, 39.53, 38.49, 36.97, 36.56, 36.20, 35.81, 31.91, 31.88, 30.69, 28.24, 28.12, 28.02, 24.29, 23.85, 22.82, 22.57, 21.05, 19.33, 18.73, 11.87. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 467.3763, Found: 467.3761.

Synthesis of CTN

The synthetic procedures for CTN are similar to those for compound 4. The residue was purified by silica gel chromatography (2:1 THF/petroleum ether) to afford the target compound CTN(0.35g, 50%) as a white solid with m.p. 145.0-146.0 °C. ^{1}H NMR ((δ ppm, 400 MHz, $CDCl_3$): 8.34-8.35 (d, 2H,), 7.87 (s, 1H), 7.56 (s, 1H), 6.36 (s, 1H), 5.69 (s, 1H), 5.30 (s, 2H), 4.86 (s, 2H), 4.41 (s, 8H), 0.67-2.45 (m, 86H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ= 155.45, 144.35, 143.89, 139.68, 139.20, 136.43, 123.04, 76.72, 76.09, 56.70, 56.66, 56.20, 50.03, 49.97, 42.32, 39.74, 39.72, 39.52, 38.54, 38.45, 36.99, 36.89, 36.56, 36.52, 36.21, 35.81, 31.90, 31.86, 31.82, 28.23, 28.15, 28.09, 28.02, 24.28, 23.89, 22.82, 22.57, 21.06, 19.34, 19.32, 18.75, 18.73, 11.87. IR (KBr, cm^{-1}): 2982, 2959, 1672. MS (ESI, m/z): Calcd for [$(M+H)^+$]: 1252.8464, Found: 1252.8460.



Scheme S1. Schematic representation of the synthesis route of compound CTN.

Determination of Detection Limit

Detection Limit of the CTN sensing system to Hg^{2+} and CTN-Hg^{2+} to OPs.

The detection limit (DL) of sensing system was determined according to the following equations:

$$s_b = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \quad (1)$$

$$S = \frac{\Delta I}{\Delta c} \quad (2)$$

$$DL = \frac{3s_b}{S} \quad (3)$$

The standard deviation (S_b) regarding present system of the aqueous solution of CTN sensing system and the instrument was determined by measuring the fluorescence intensities (x_i) of the solution for more than 10 times, and calculating the corresponding average intensity ($\bar{x}$) firstly. By fitting the intensity data and the average intensity as obtained into equation 1, the value of the standard deviation (S_b)

was obtained.

Then, a certain amount of Hg^{2+} ion was added into the ethanol solution of CTN, and then the fluorescence emission intensities were recorded. Corresponding variations in intensity (ΔI) and those in phenol concentration (Δc) were calculated. By fitting the data into equation 2, S value for the present system was obtained. Finally, with the values of S_b and S as determined, the DL for the system was calculated according to equation 3.

The detection limit (DL) of CTN-Hg^{2+} sensing system to OPs according to the same method.

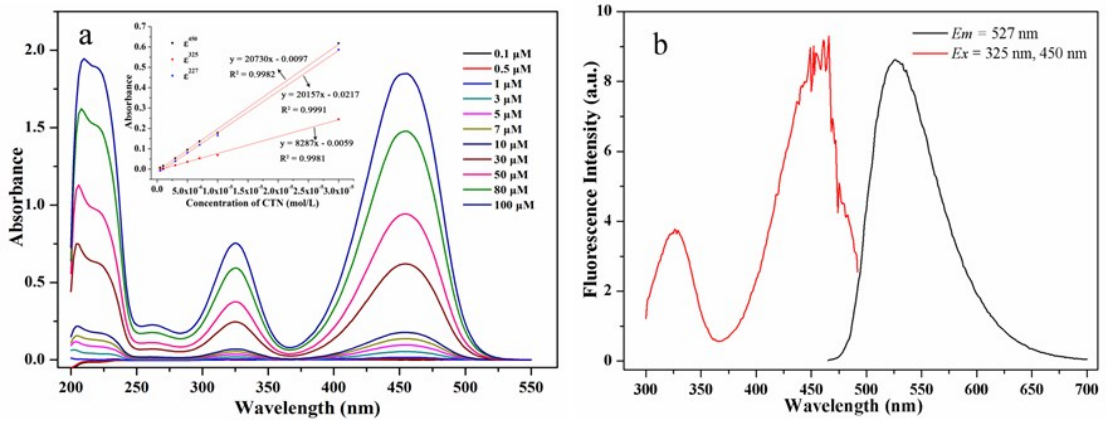


Fig. S1 (a) UV-vis absorption spectra of the ethanol solution of CTN recorded at different concentrations. Inset: The plots of UV-vis absorption of CTN in ethanol as a function of the fluorophore concentration. (b) Steady-state fluorescence excitation and emission spectra of CTN in ethanol.

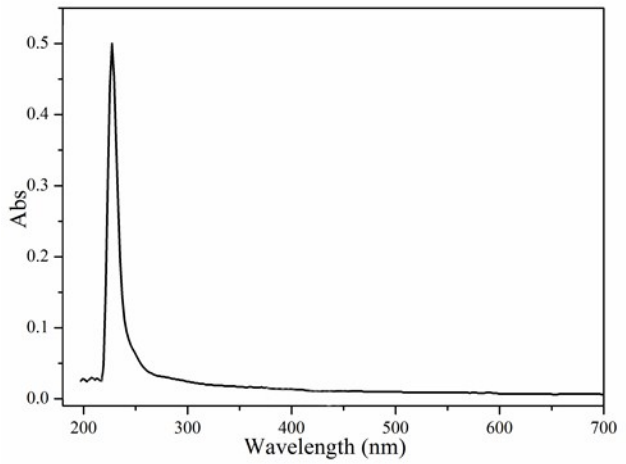


Fig. S2 UV-vis absorption spectra of intermediate 4 in DCM solution (1×10^{-5} mol/L).

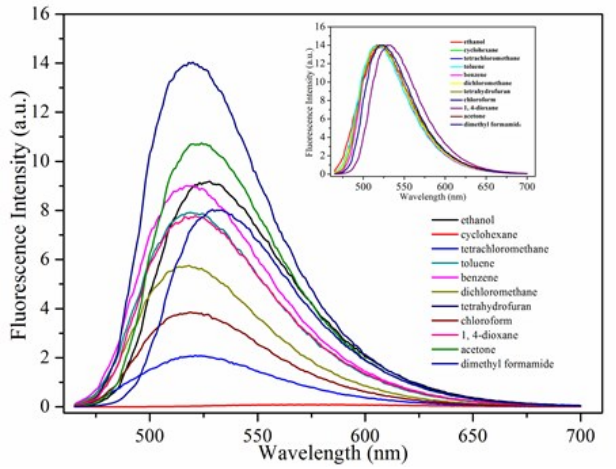


Fig. S3 Fluorescence emission spectra of CTN recorded in its solutions of different solvents at a concentration of 1×10^{-5} mol/L (λ_{ex} = 450 nm).

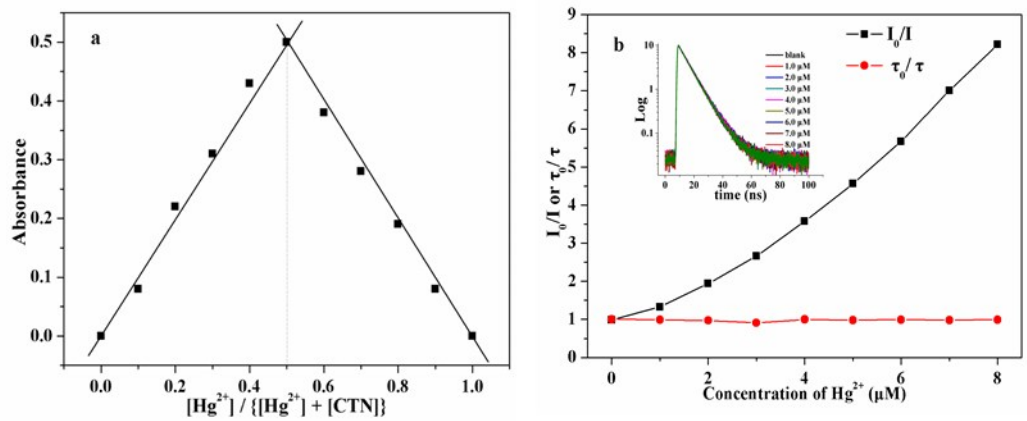


Fig. S4 (a) Job's plot for 1 : 1 complex of CTN probe and Hg^{2+} ion at 450 nm in EtOH/H_2O (v/v=10:1, 10 mM HEPES buffer, pH 7.4) solution; (b) Stern-Volmer plots of I_0/I and τ_0/τ against the concentration of Hg^{2+} (λex/λem = 450/527 nm).

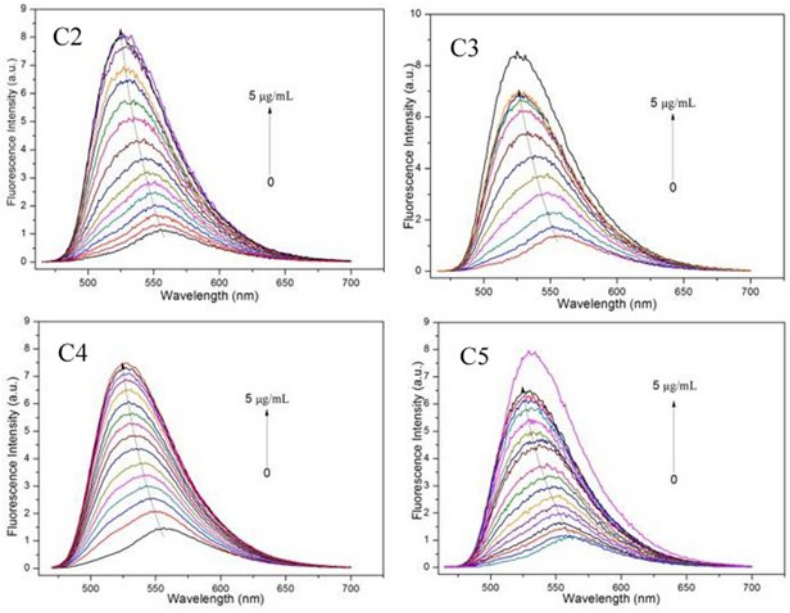


Fig. S5 Fluorescence emission intensity changes of CTN-Hg^{2+} sensing system in EtOH/H_2O (v/v=10:1, 10 mM HEPES buffer, pH 7.4) with 0–5 μg/mL addition of analyte.

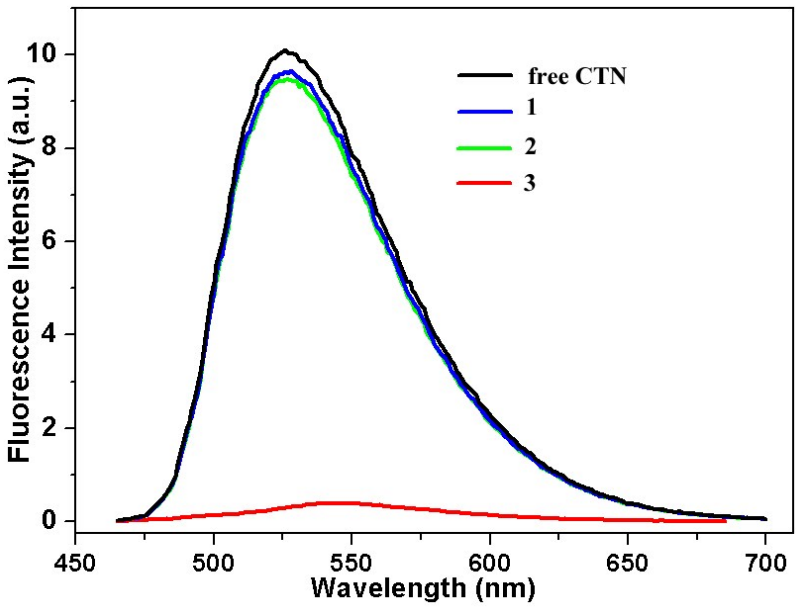


Fig. S6 Fluorescence emission changes of CTN (10 μM) sensing system in EtOH/H_2O (v/v=10:1, 10 mM HEPES buffer, pH 7.4) upon addition of 1.0 equivalent of OPs, then 1.0 equivalent of Hg^{2+}, and then another 2.0 equivalent of Hg^{2+}. Note: 1-CTN + OPs, 2-1 + Hg^{2+} (1.0 equiv.), 3-2 + Hg^{2+} (1.0 equiv.).

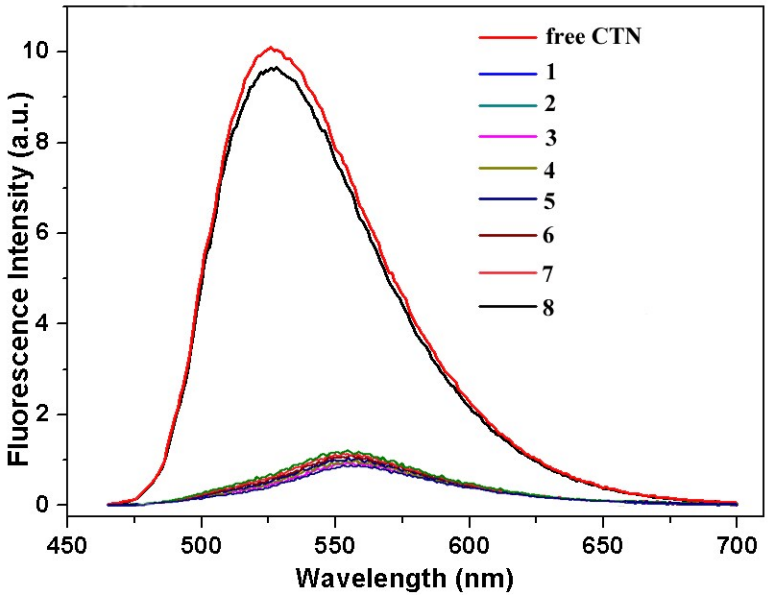


Fig. S7 Fluorescence emission spectra of CTN in the absence and presence of other chemicals. Note: 1-CTN+Hg^{2+} (one equivalent), 2-1 + cyfluthrin, 3-2 + carbofuran, 4-3 + carbendazim, 5-4 + cymperator, 6-5 + $H_2PO_4^-$, 7-6 + HPO_4^{2-}, 8-7 + OPs.

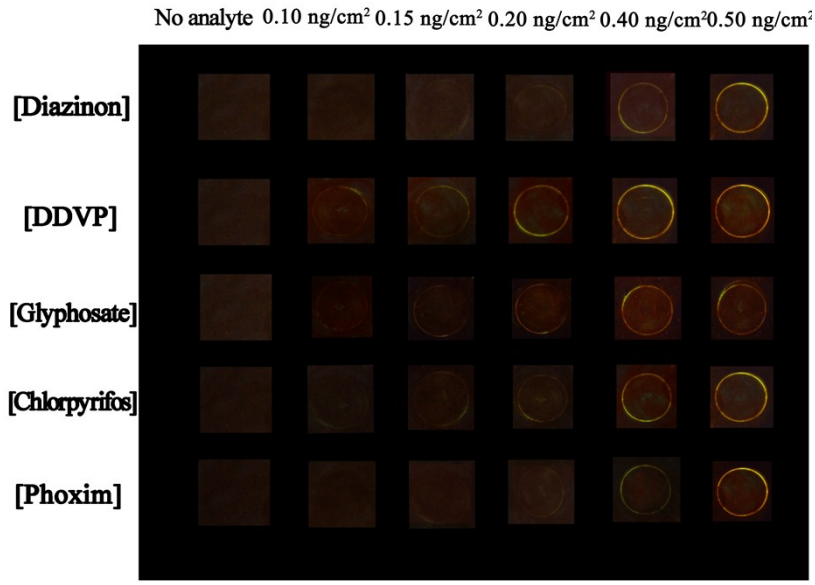


Fig. S8 Picture taken under excitation at 365 nm of a TLC plate, which was pre-treated by the CTN-Hg^{2+} complex, upon introduction of different amount of the OPs under test.

Table S1. Comparison of different fluorescent probes for OPs detection.

Method	Detection limit (DL) (mg/L)					Reference
	Glyphosate	DDVP	Chlorpyrifos	Diazinon	Phoxim	
Intramolecular Indicator Displacement Assays (IIDAs) sensor	0.2					Pavel et al.,[1]
Quantum dots/bi-enzyme sensor		0.000992				Tang et al.,[2]
QDs-SA-cFLISA sensor			0.0038			Ning et al.,[3]
This work	0.015	0.018	0.087	0.098	0.113	

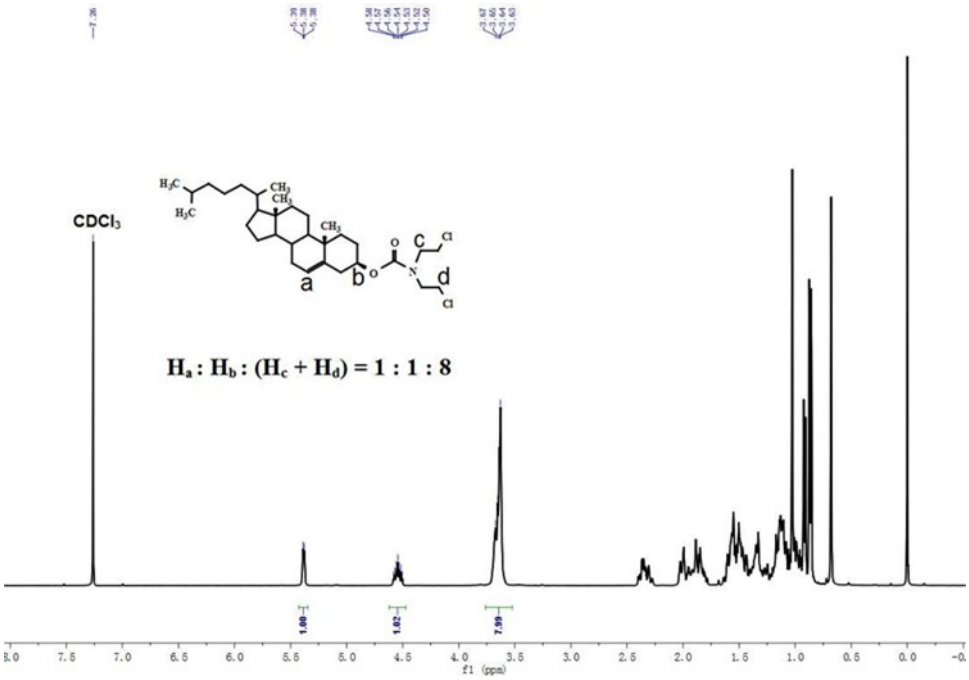


Fig. S9 ^{1}H NMR spectrum of I-2

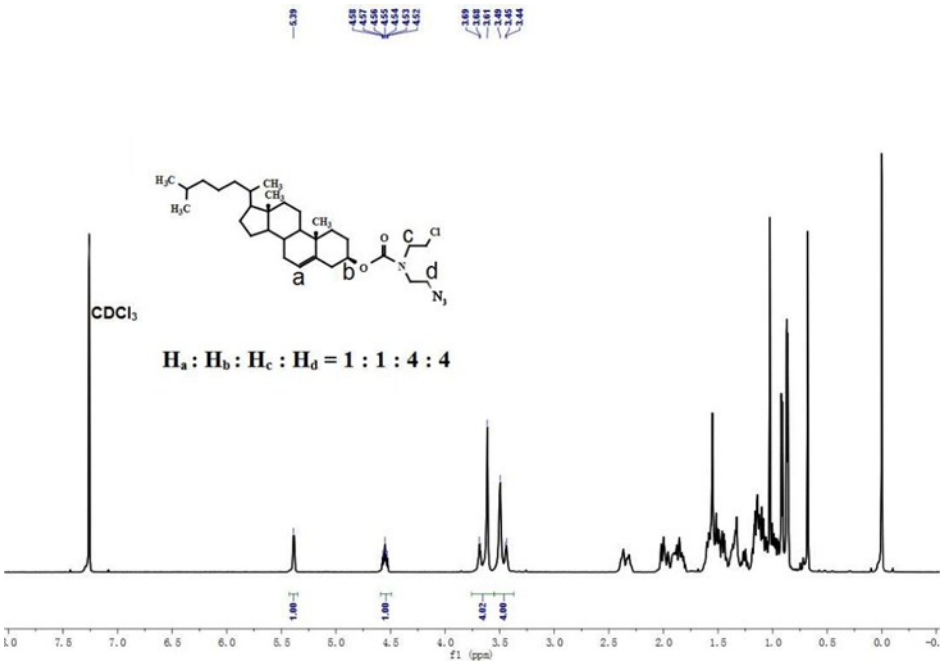


Fig. S10 ^{1}H NMR spectrum of I-3

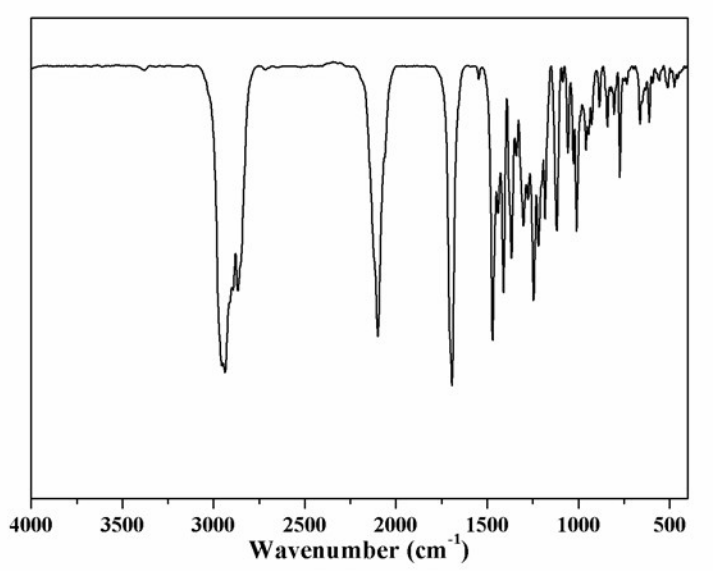


Fig. S11 FT-IR spectrum of I-3

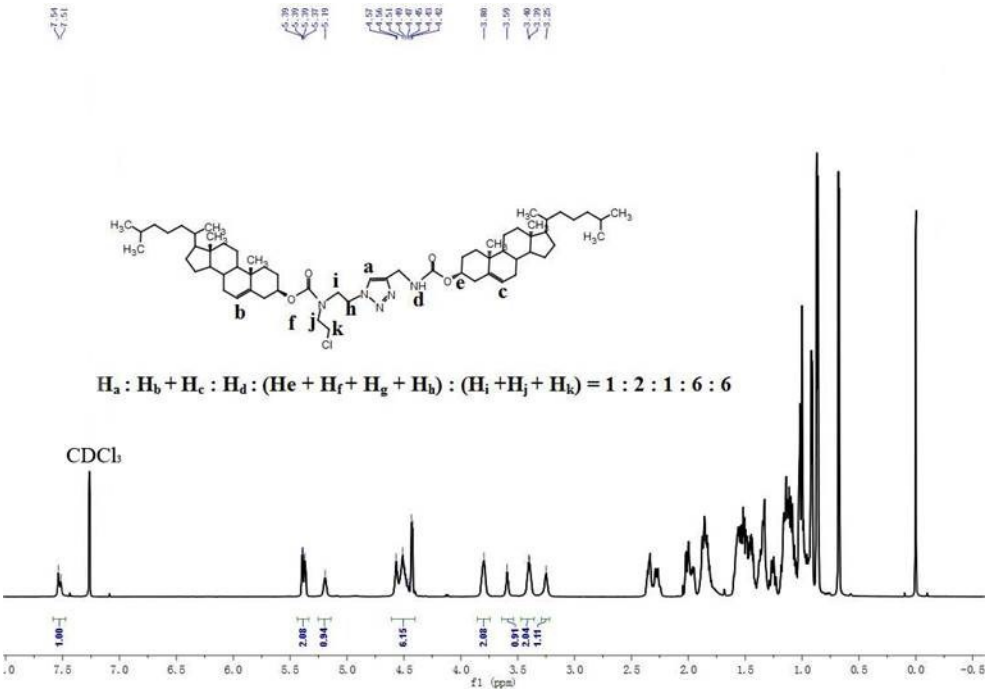


Fig. S12 ^{1}H NMR spectrum of I-4

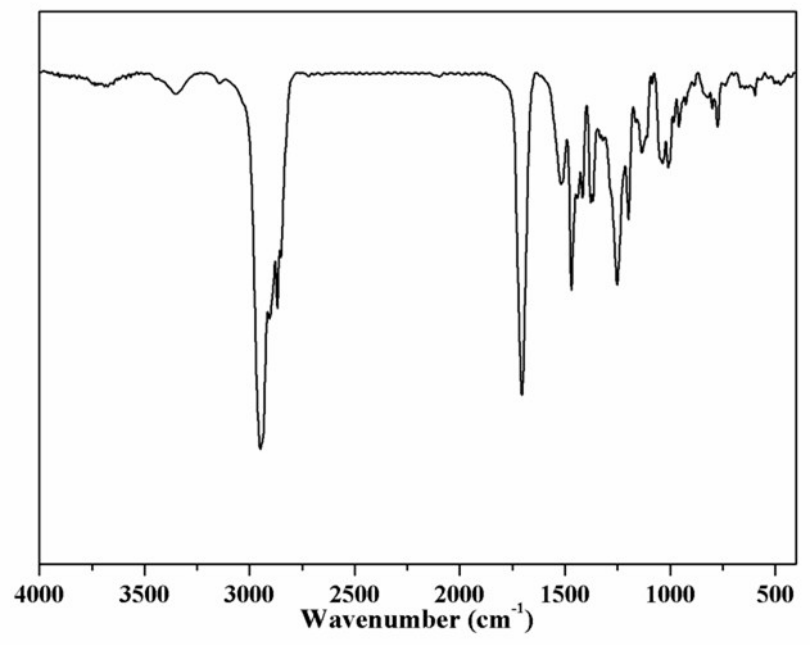


Fig. S13 FT-IR spectrum of I-4

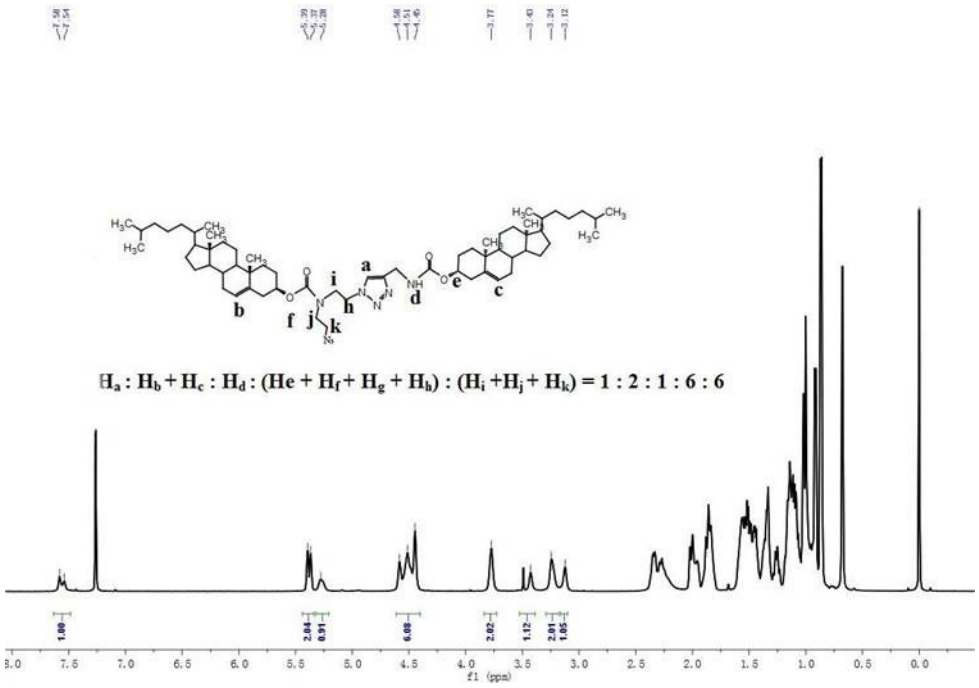


Fig. S14 ^{1}H NMR spectrum of I-5

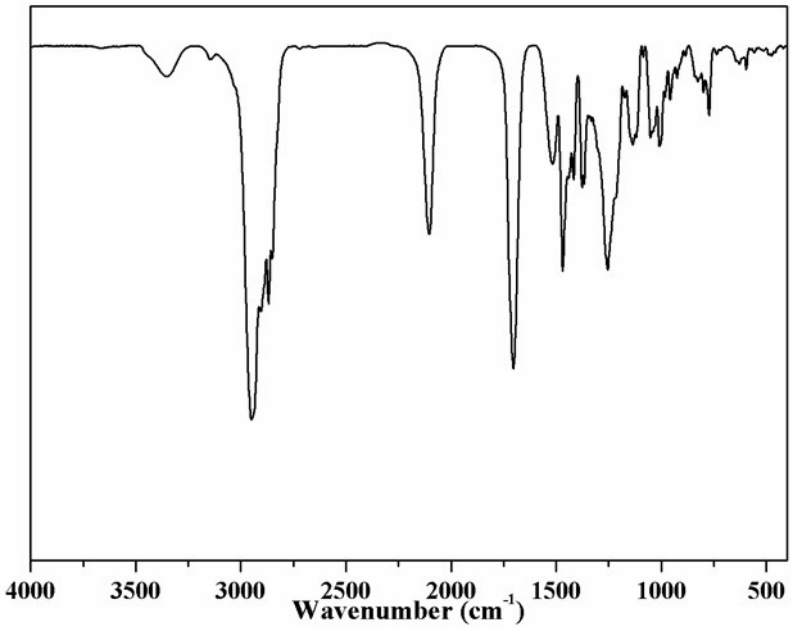


Fig. S15 FT-IR spectrum of I-5

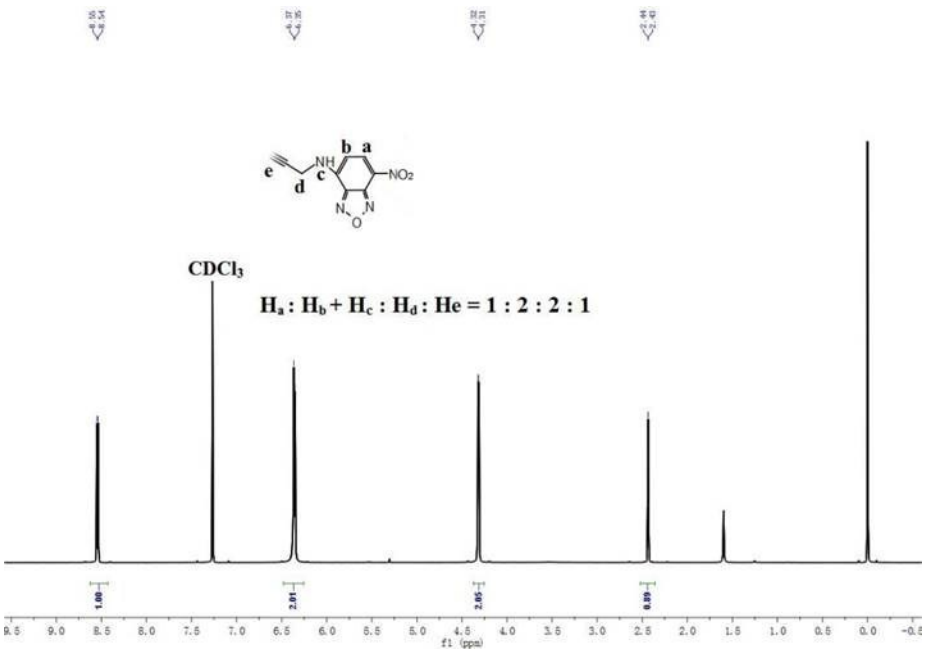


Fig. S16 ^{1}H NMR spectrum of I-6

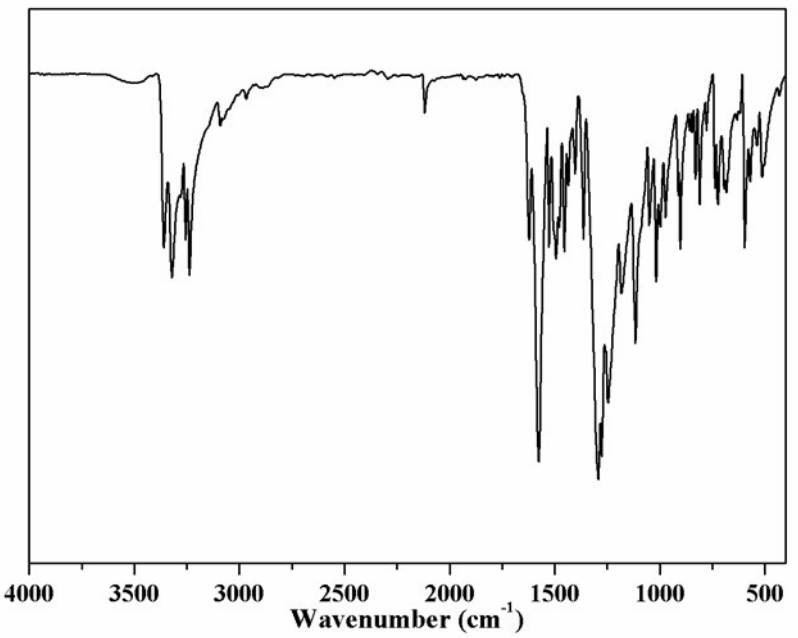


Fig. S17 FT-IR spectrum of I-6

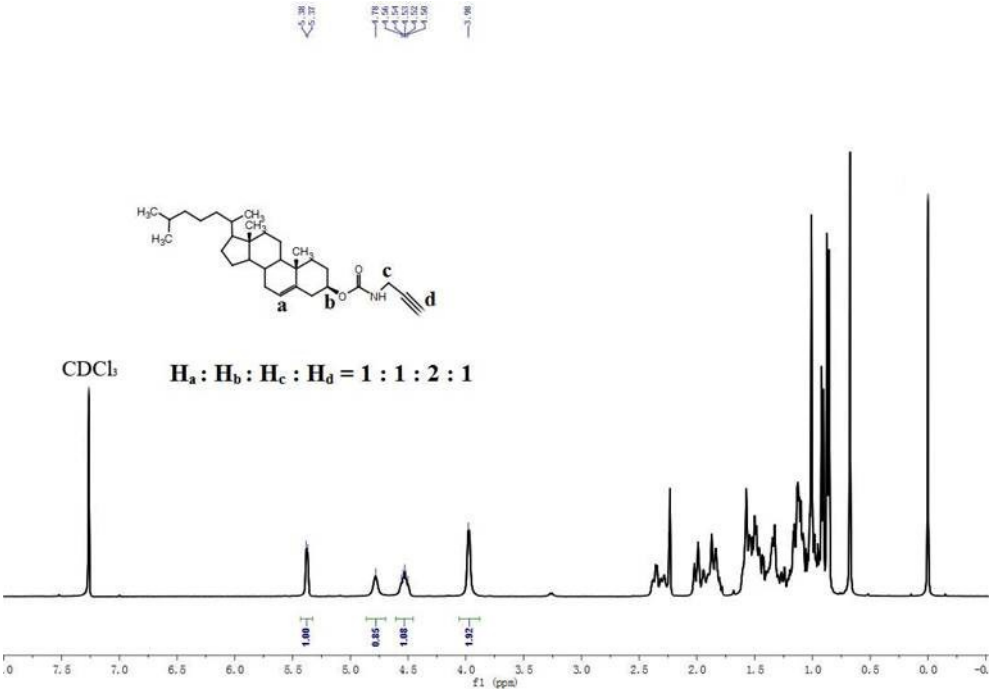


Fig. S18 ^{1}H NMR spectrum of I-7

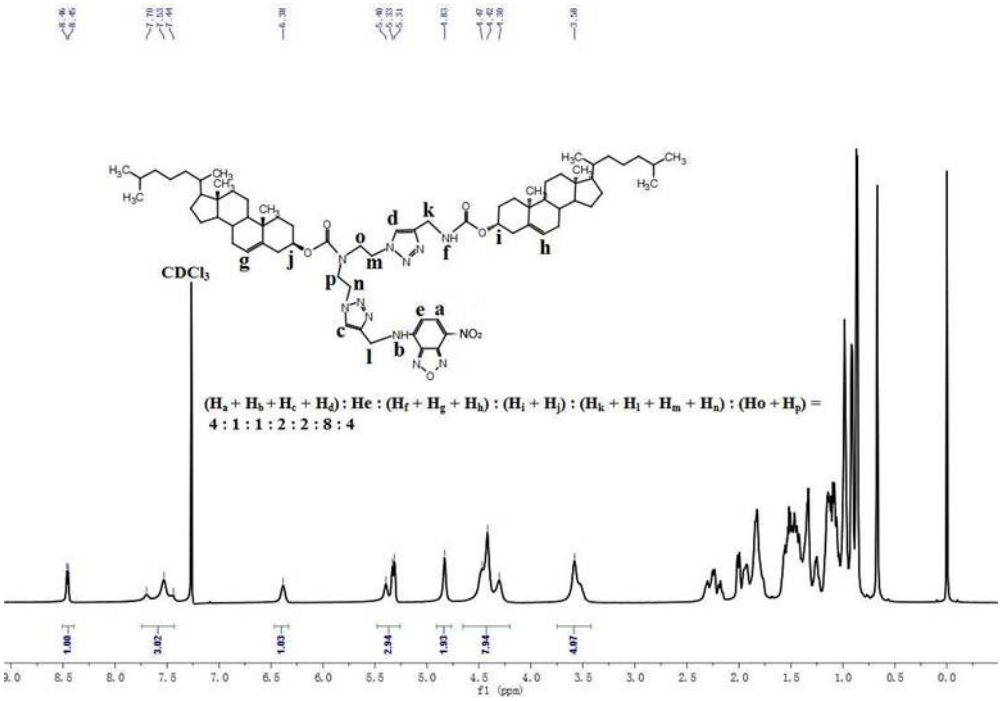


Fig. S19 ^{1}H NMR spectrum of CTN

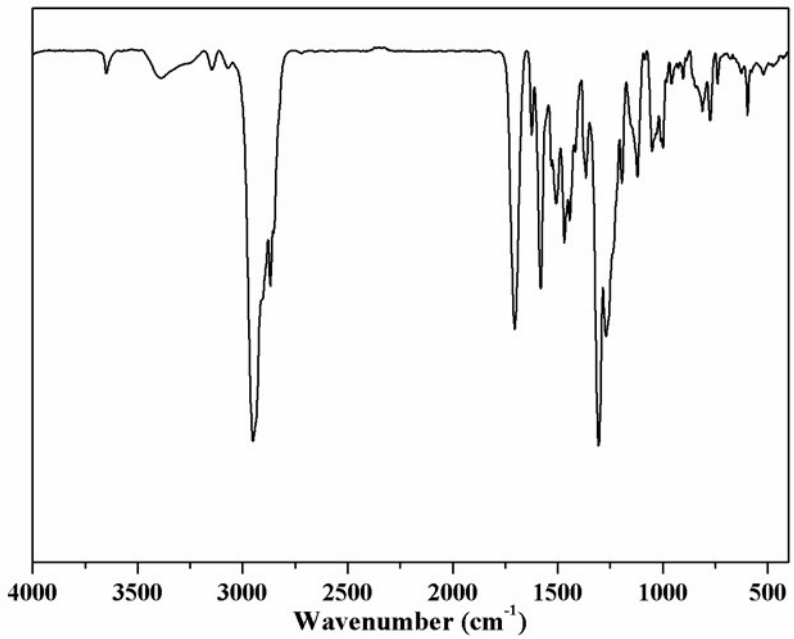


Fig. S20 FT-IR spectrum of CTN

References:

1. T. Minami, Y. L. Liu, A. Akdeniz, P. Koutnik, N. A. Esipenko, R. Nishiyabu, Y. Kubo, P. A. Jr. *J. Am. Chem. Soc.,* 2014, **136**, 11396-11401.
2. X. W. Meng, J. F. Wei, X. L. Ren. J. Ren, F. Q. Tang, *Biosens. and Bioelectron.,* 2013, **47**, 402-407.
3. Y. P. Chen, H. L. Ren, N. Liu, N. Sai, X. Y. Liu, Z. Liu, Z. X. Gao, B. A. Ning, *J. Agric. Food Chem.,* 2010, **58**, 8895-8903.